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Primer

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Autoimmune haemolytic anaemias

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Abstract

Adult autoimmune haemolytic anaemias (AIHAs) include different subtypes of a rare autoimmune disease in which autoantibodies targeting autoantigens expressed on the membrane of autologous red blood cells (RBCs) are produced, leading to their accelerated destruction. In the presence of haemolytic anaemia, the direct antiglobulin test is the cornerstone of AIHA diagnosis. AIHAs are classified according to the isotype and the thermal optimum of the autoantibody into warm (wAIHAs), cold and mixed AIHAs. wAIHAs, the most frequent type of AIHAs, are associated with underlying conditions in ~50% of cases. In wAIHA, IgG autoantibody reacts with autologous RBCs at 37 °C, leading to antibody-dependent cell-mediated cytotoxicity and increased phagocytosis of RBCs in the spleen. Cold AIHAs include cold agglutinin disease (CAD) and cold agglutinin syndrome (CAS) when there is an underlying condition. CAD and cold agglutinin syndrome are IgM cold antibody-driven AIHAs characterized by classical complement pathway-mediated haemolysis. The management of wAIHAs has long been based around corticosteroids and splenectomy and on symptomatic measures and non-specific cytotoxic agents for CAD. Rituximab and the development of complement inhibitors, such as the anti-C1s antibody sutimlimab, have changed the therapeutic landscape of AIHAs, and new promising targeted therapies are under investigation.

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Introduction

Autoimmune haemolytic anaemias (AIHAs) are a group of anaemias mediated by the presence of autoantibodies targeting one or several autologous membrane antigens expressed on red blood cells (RBCs) leading to the accelerated destruction of RBCs. They are often viewed as a 'benign disease' compared with haematological malignancies; however, AIHAs may be life-threatening and are associated with a high disease burden, including an increased risk of thrombosis[1]. AIHAs are classified into warm (wAIHA), cold (cAIHA) or mixed AIHA based on the immunochemical properties of the autoantibody and on the optimal temperature at which it binds to RBCs[1,2] (Table 1). The distinction between wAIHA and cAIHAs is a crucial step of the diagnostic procedure as it influences both the management and outcome[1,3]. wAIHAs represent ~70% of all AIHAs in adults, whereas cAIHAs and mixed AIHAs respectively account for 15–20% and 7–8% of adult AIHAs[4]. At least 50% of adult wAIHAs, defined as 'secondary', are associated with an underlying disease[1,3] (Box 1), whereas cAIHAs are divided into primary chronic cold agglutinin disease (CAD), a well-characterized indolent B cell clonal disorder, and cold agglutinin syndrome (CAS), which encompasses rare cases of cAIHAs secondary to malignancies or acute infection[1,5]. The diagnosis of AIHA relies on the positivity and interpretation of the direct antiglobulin test (DAT), associated with the search and titration of cold agglutinin in serum whenever the presence of complement (C3d) on the surface of RBCs is detected by the DAT[1]. Treatment of AIHAs has long been mostly empirical and based on limited evidence derived from expert opinion and retrospective studies. In the past two decades, the management of AIHAs benefited from the development of more specific therapies for lymphoproliferative disorders and other autoimmune diseases, and several prospective studies have been conducted both in wAIHAs and CAD[1,5].

In this Primer, which focuses on adult AIHAs (unless otherwise specified), the diagnostic procedure and classification of AIHAs are addressed, and insights into the epidemiology, pathophysiology and management of both wAIHA and CAD, which are the two major types of AIHAs, are provided. Lastly, current developments, unmet needs and future directions are also discussed.

Epidemiology

Global incidence rates

The exact incidence of AIHAs is unclear given the high heterogeneity of the conditions. In fact, AIHAs may show a different epidemiology according to age group (that is, paediatric versus adult onset AIHAs), thermal subtypes (that is, cold versus warm versus mixed or atypical forms), and primary versus secondary disease[5] (Table 2). Overall, the incidence of AIHAs regardless of type was calculated to be 1.8 per 100,000 person-years in a nationwide, registry-based Danish study[6], and 2.4 per 100,00 person-years in a French study[7]. Other global estimates range from 0.8 to 3 cases per 100,000 person-years for all AIHAs and a prevalence of ~17 per 100,000 person-years, of which 60–75% were wAIHA, 15–25% were cAIHA and the remaining were atypical forms, including mixed disorders and DAT-negative AIHAs[1,5]. Regarding thermal subtypes, wAIHA can occur at any age but is more frequent in older ages and in women. The French epidemiology study reported an incidence 10 times higher in people aged ≥75 years compared with that in people aged <50 years[7]. Data on cAIHA, and especially CAD, reports a median age of 67–76 years according to different reports, with a slight female predominance[1,8,9].

The epidemiology of AIHAs is highly variable depending on geography and climate[9]. A study of 232 patients from Norway and Italy found an overall prevalence of 20 per million persons and an incidence of 1.9 per million persons per year, which lowered to 5 per million persons and 0.5 per million persons per year, respectively, if considering only Italy[9]. A seasonality in diagnosis and mortality of CAD, with more events observed during the coldest seasons, was also reported[10].

Finally, the incidence of DAT-negative AIHA (generally around 5% of all AIHAs) is variable depending on the diagnostic criteria adopted and the sensitivity and specificity of available tests[11]. It is worth mentioning that the incidence and prevalence of clinically defined AIHAs should be distinguished from that of 'positive DAT'. The latter may be found in up to one in 10,000 healthy blood donors[2] and up to 100 times more frequently in hospitalized patients, especially among those with lymphoproliferative disorders. The significance of a weakly positive DAT found in a healthy individual with no previous history of transfusion and no features of haemolysis is uncertain, but there is no evidence to recommend long-term surveillance in such cases. Additionally, by highly sensitive methods, anti-RBC autoantibodies may be found in chronic lymphocytic leukaemia (CLL)[12] and, to a lesser degree, in myelodysplastic syndromes and primary myelofibrosis[13,14] but with only a few cases developing clinical haemolysis.

Most epidemiology data is reported from Western countries, and little is known about geographic or ethnic variations. In Asia, in

Table 1 | Classification and main characteristics of AIHA subtypes

Biological feature	wAIHA	cAIHA: CAD or CAS	Mixed AIHA	PCH
Autoantibody isotype	IgG (mostly IgG1 and IgG3); rarely IgA or IgM	IgM (monoclonal in CAD or polyclonal in some CAS)	IgG+IgM	IgG (Donath–Landsteiner biphasic haemolysin)
Thermal optimum	37 °C	4 °C	4 °C or large amplitude (4 °C to 30 °C)	>30 °C[a]
DAT pattern	IgG±C3	C3[b]	IgG+C3	C3[c]
Cold agglutinin	Absent	High titre >1/64	Titre >1/64	Absent
Eluate	IgG	Non-reactive	Reactive	Non-reactive
Antigen specificity	Often polyspecific (Rh, glycophorin 3, protein band 3)	I > i	Panreactive I > i	P

AIHA, autoimmune haemolytic anaemia; cAIHA, cold AIHA; DAT, direct antiglobulin test; CAD, cold agglutinin disease; CAS, cold agglutinin syndrome; PCH, paroxysmal cold haemoglobinuria; Rh, rhesus; wAIHA, warm AIHA. [a]Binds optimally at cold temperatures and then causes rapid intravascular haemolysis of red blood cells when warmed to 37 °C. [b]DAT pattern may be also weakly positive for IgG in ~15–20% of cAIHAs. [c]5–10% of cases may also show weakly positive IgG.

Box 1 | Main conditions associated with secondary adult warm autoimmune haemolytic anaemias

Lymphoproliferative disorders[a]
Chonic lymphocytic leukaemia
Marginal-zone lymphoma and other B cell lymphomas
Large granular lymphocytic leukaemia
Monoclonal B cell lymphocytosis
Angio-immunoblastic T cell lymphoma
Hodgkin lymphoma

Solid tumours
Thymoma
Castleman disease
Rosai–Dorfman histiocytosis
Ovarian dermoid cyst
Carcinoma

Autoimmune/inflammatory diseases[a]
Systemic lupus erythematosus
Sjögren disease
Rheumatoid arthritis
Antiphospholipid syndrome
Ulcerative colitis, Crohn's disease
Autoimmune hepatitis
Sarcoidosis
Eosinophilic fasciitis
IgG4-related disease
Relapsing polychondritis

Infections
Viruses: Ebstein–Barr virus, cytomegalovirus, parvovirus B19, SARS-CoV-2, HIV
Bacteria: tuberculosis, brucellosis, *Bartonella*, syphilis
Parasites: babesiosis

Primary immunodeficiencies/inborn errors of immunity
Common variable immunodeficiency
IgA deficiency
Autoimmune lymphoproliferative syndrome
LRBA deficiency, CTLA4 insufficiency, STAT3 gain-of-function mutation

Miscellaneous
Myelodysplasia
Chronic myelomonocytic leukaemia
Bone marrow or stem cell transplantation
Drugs
Checkpoint inhibitors

[a]Other autoimmune diseases, such as pernicious anaemia or autoimmune thyroiditis, are other autoimmune conditions occurring on a predisposing genetic background rather than true secondary adult warm autoimmune haemolytic anaemias.

a study of 79 patients from a referral centre in India[15], the median age of patients with AIHA was 30.4 years (range 0.2–66 years), which is younger than in ages reported in higher-income countries; 63% were women and 34% had a secondary AIHA. Similar data were reported in another study from a tertiary care hospital in India, which focused on the characterization of 43 patients with AIHA with a median age of 31 years (range 12–70 years) with a male-to-female ratio of 1:3.3 (ref. 16). For CAD, a registry-based study of 344 patients from Japan reported similar demographic features as patients in Europe and the USA, with a slight female predominance (53%) and median age at onset of 66.8 years[17]. A study from the 1970s of 100 patients with AIHA in Thailand[18] also reported a younger age, a rarer occurrence of cAIHA, and a higher prevalence of mixed autoantibodies (>50% of cases) compared with white people; however, this data is old and should be reproduced for an updated insight into AIHA in Thailand. Data from Latin America are even less represented, and mainly related to the epidemiology of secondary AIHAs. In Africa, a retrospective study conducted on 3,182 consecutive Moroccan patients (2,183 women and 999 men) whose sera were tested for 14 autoantibody profiles between 2010 and 2016 found an AIHA prevalence of 0.4%[19]. Another study from a referral centre in Nigeria reported only 13 patients with AIHA over a 10-year follow-up, with a median age of 42 years at diagnosis and 42% having secondary forms[20]. Altogether, although data may reflect differences in diagnostic capacities, these data suggest a lower incidence of AIHA in low-income countries as well as AIHAs associated with a younger age compared with high-income countries.

Predisposition for AIHA

Most of the studies investigating predisposing genetic factors for developing AIHA were performed in paediatric cohorts of Evans syndrome, defined by the concomitant or sequential occurrence of AIHA and immune thrombocytopenia with or without autoimmune neutropenia. Pathogenic variants were detected in up to 40% of paediatric cases of Evans syndrome, mostly involving genes related to primary immunodeficiencies[21]. The main distinguishing features of childhood AIHA are summarized in Box 2. In adulthood, genetic predisposition is likely to be lower and only a few studies have reported an association with genes involved in antigen recognition (that is, specific HLA loci; stereotyped rearrangements of genes encoding the variable region of the immunoglobulin chains), T and B cell maturation and proliferation, and cytokine homeostasis (CTLA4 and lymphotoxin-α) polymorphisms[22].

An underlying associated condition can be found in ~50% of adults with wAIHAs (Table 2), with lymphoproliferative disorders (~30% of the causes, mainly CLL 5–11%) and connective tissue diseases (mainly systemic lupus erythematosus (SLE) 10%) being the most frequent conditions[3,4,23,24]. wAIHAs and, to a lesser extent, cAIHAs may also be triggered by some viral infections, particularly parvovirus B19 (in up to 20% of cases), hepatitis C, hepatitis B and hepatitis A virus, and more rarely HIV[25]. Moreover, several AIHA cases developing during or after SARS-CoV-2 infection or vaccination have been reported since 2020 (refs. 5,26). Regarding bacteria, CAS may be classically induced by *Mycoplasma pneumoniae* infection[27] and, more rarely, by tuberculosis[28].

Table 2 | Epidemiology of adult AIHAs

	All types of AIHAs	wAIHA	CAD
Prevalence per 100,000 (country)		17.01 (95% CI 15.96–18.12)[6] (Denmark)	1.26 (Denmark)[8], 0.5 (Norway)[4]
Incidence per 100,000 person-years (country)	1.8 (95% CI 1.66–1.89)[6] (Denmark), 2.44 (95% CI 2.39–2.48) (France)[7]	No data	0.18 (95% CI 0.15–0.22)[8] (Denmark), 0.19 (Norway)[9], 0.048 (northern Italy)[9]
Median age (years) at diagnosis (min–max or IQR)	68.7 (IQR 53.8–78.3)[6], 69.5 years (min–max: 51–81)[7]	58 years[4]	Mean age 67 years[9] (Norway and Italy), 72.2 (IQR 20.3–89.6) (Denmark)[8]
Sex ratio (percentage of women)	55%[7] to 64%[4]	50%[11] to 55%	56%[9] to 58%[8]
Incidence of thrombosis	The 1-year cumulative incidence of hospitalization for thrombosis 6.0% (95% CI 5.6–6.5)[7]	No data on 1-year cumulative incidence (rate of thrombosis 20–23% in retrospective series)[25,80]	11.5% at 5 years; overall risk of thrombotic events; adjusted HR 1.94 (95% CI 1.64–2.30)[8]
Mortality	1-year survival and median survival of 82.7% and 9.8 years for primary wAIHA, 69.1% and 3.3 years for secondary wAIHA[37], and 85.5% and 8.8 years for CAD[37]	Mortality of 8%[25] and 21%[4] after a mean follow-up of 3.8 and a median follow-up of 2.8 years	Mortality at 1 and 5 years: 17% and 39% (versus 3% and 18% in the comparison cohort)[8]; median estimated survival from diagnosis: 16 years (0–32); 5-year survival: 83%[4]

AIHAs, autoimmune haemolytic anaemias; CAD, cold agglutinin disease; IQR, interquartile range.

Drug-induced immune haemolytic anaemia (DIIHA) is rare (Box 3), with an estimated annual incidence of 1 in 1 million of the population[29], although it might be underestimated given the reporting of severe haemolytic events only. Anti-drug antibodies have been distinguished in drug-independent and drug-dependent antibodies. By definition, drug-independent antibodies do not require the addition of the drug into in vitro test systems for their detection and are therefore authentic anti-erythrocyte antibodies detected by means of the DAT, thus indistinguishable from those causing AIHA. The number of drugs associated with DIIHA has progressively increased (13 in 1967, 32 in 1980, and 125 in 2007)[29,30]. Associated drugs include neuroleptics (that is, historical α-methyldopa), antimicrobials (penicillin and cephalosporins), anti-inflammatories and antineoplastics[29]. The latter encompass purine analogues, such as fludarabine, but also novel targeted biologic treatments such as checkpoint inhibitors[31].

Morbidity and mortality

In the Italian GIMEMA cohort of 308 patients with primary AIHA, mortality was reported at 20% during a 4-year follow-up, and 3.6% of deaths were considered as related to AIHA or its complications: mainly infections, thrombosis (myocardial infarction and pulmonary embolism) or multiorgan failure[4]. Prognostic factors included lower haemoglobin at onset (<8 g/dl), presence of Evans syndrome, infections and acute renal failure[32], whilst AIHA type had no significant effect on prognosis. In wAIHA, mortality ranges from 3% to 20%, mainly associated with infections, malignancies and thrombotic events[4,24,33]. Additionally, a link with active haemolysis has been reported[24]. In the largest series of patients with CAD, the mortality at 5 years ascribed to CAD or its complications was of 3.5%[9]. Finally, it is worth mentioning that IgM wAIHA are generally very severe and difficult to diagnose (often DAT negative), and mainly show a dismal fulminant course[33].

The presence of an associated condition can affect prognosis, particularly in the context of malignancies. Two ICD code registry-based studies compared AIHA mortality with a control group matched for age and sex. In the French cohort, the adjusted hazard risk of 1-year mortality was 2.9 (95% CI 2.7–3.1) for primary AIHA, 3.5 (95% CI 3.2–3.8) for AIHA secondary to haematological malignancies and 4.6 (95% CI 3.9–5.6) for other secondary forms[7]. In the Danish cohort, adjusted hazard risks were 6.5 (5.4–7.9) and 4.9 (4.1–5.9) for primary and secondary AIHA, respectively, and 3.2 (1.6–6.1) for CAD[34]. Notably, hazard ratios were maximal within the first 100 days after AIHA diagnosis and decreased during follow-up. Among causes of death, thromboembolic or cardiac events represented 2.8% of fatalities in the first 100 days; other significantly increased causes of mortality were infections and bleeding[34].

Two French cohorts of patients with AIHA admitted to the intensive care unit (ICU) reported mortality as high as 30% mainly due to multiorgan failure[35]. In another study, 13% of patients died in the ICU after a median of 3.5 days from ICU admission due to cardiac arrest secondary to refractory AIHA (5 patients) and massive pulmonary embolism (3 patients)[36]. It is important to note that 92% of this cohort displayed inadequate reticulocytosis (an increase in reticulocytes, immature RBCs), which is defined by a bone marrow responsiveness index of <121, likely contributing to more severe anaemia. Nine patients died within 1 year, of whom 6 had ongoing haemolysis at time of death[36].

AIHAs following allogenic stem cell or bone marrow transplantation are also often severe and associated with a high rate of complications, including graft failure and increased mortality[37].

AIHA in pregnancy

Based on the data from a multicentre retrospective study in Europe on 45 pregnancies in 33 women who had been diagnosed with AIHA[38], the risk of relapse or AIHA worsening during pregnancy was estimated to be of ~50%. A complete response to treatment (mostly corticosteroids) was observed in two-thirds of patients and a transfusion of packed RBCs was initially needed in 50%. Maternal or fetal adverse outcomes occurred in 25% of the cases and fetal complications were more frequent than expected in healthy women but transient neonatal AIHA was observed in only one case[39].

Mechanisms/pathophysiology

wAIHA

Mechanisms of haemolysis. wAIHA is an autoimmune disease in which haemolysis is induced by polyclonal autoantibodies directed towards one or several antigens expressed on RBC membranes with a maximal binding affinity at 37 °C. Whereas the mechanisms ultimately leading to the accelerated destruction of RBCs, namely

antibody-dependent cellular phagocytosis, antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), are well established[1,5,40] (Fig. 1), the upstream mechanisms involved in the loss of tolerance towards autologous RBCs are far from being fully elucidated. Some of these mechanisms are extrapolated from mice models of AIHAs, such as NZB mice[41], as studies in humans remain limited given the rarity of the disease.

Autoimmune response. In patients with inborn errors of immunity (IEI), the relative risk of developing AIHA is highly increased when compared to the general population[42]. AIHA alone or in the setting of Evans syndrome is indeed a common manifestation of IEI affecting various immune pathways such as apoptosis[43], T cell regulation[44] or cytokine signalling[45], highlighting the fact that several non-redundant mechanisms are necessary to avoid autoimmune responses. Interestingly, the germline immunoglobulin gene repertoire contains sequences coding for heavy chains reactive with RBC antigens such as the *IGHV4-34* gene[45,46]. The onset of AIHA after infectious events[5] or drug exposure[47] also suggests that an inflammatory milieu, molecular mimicry or drug–RBC neoantigens can trigger an autoimmune reaction against RBCs. In healthy individuals, the spleen is the main organ involved in RBC turnover, which relies mostly on the action of red pulp macrophages settled into splenic microcirculatory beds recognizing, filtering out and degrading senescent or structurally altered RBCs[48]. In wAIHA, the spleen is the main site of haemolysis through the accelerated phagocytosis of opsonized RBCs bound to the Fcγ receptor (FcγR) expressed on splenic macrophages[39]. The spleen is also a source of local autoantigens and the site of adaptive immune response, leading to autoantibody production and ultimately ADCC[39]. It has been shown that splenic tissues from patients with wAIHA display a high number of germinal centres, that in turn generate autoreactive plasma cells producing anti-RBC autoantibodies, which ultimately enter peripheral circulation[49]. Follicular responses require T cell help and, accordingly, rhesus peptides may stimulate in vitro proliferation of circulating or splenic mononuclear cells from patients with AIHA in a major histocompatibility complex (MHC) class II-dependent manner[50,51]. The produced autoantibodies, which are mainly of IgG1 and IgG3 isotypes[1,52], can target some RBC membrane proteins, especially band 3 protein, glycophorin A and some antigens from the Rhesus system[53,54]. IgG1 and IgG3 may also directly activate the classical complement pathway, leading to CDC towards RBCs into the vasculature (Fig. 1). A shift towards a T helper 17 (T_H17) polarization of T effector $CD4^+$ T cells has been observed in wAIHA in several studies[55,56], and IL-17 concentrations were found to be elevated in patients and correlated to haemolysis intensity[55]. $CD4^+$ T regulatory (T_{reg}) cells have been studied in the context of AIHA, with reports of clones specific for RBC antigens[57] but also decreased numbers and function of T_{reg} cells in patients with AIHA[56,58], with a suggested role of simultaneous TNF increase. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a monogenic disorder due to loss-of-function mutation in the gene encoding the forkhead box P3 (FOXP3) transcription factor, which is crucial for the development, maturation and maintenance of T_{reg} cells. Interestingly, it seems that about a third of patients diagnosed with IPEX syndrome develop AIHA, providing strong evidence for the role of T_{reg} cell dysfunction in the loss of tolerance towards autologous RBCs[59]. Nonetheless, AIHA is a frequent condition also in various IEI such as RAG1 or RAG2 deficiencies, activated PI3K delta syndrome, and autoimmune lymphoproliferative syndrome. The dysregulation of other cytokines in serum or in culture from patient cells has been described, including elevations of TGFβ, IL-2, IL-4, IL-6 and IL-21 or a decrease in IFNγ, reflecting an inflammatory milieu and a skewed T_H polarization in AIHA[56,60]. Alteration of cytokine polymorphisms has been reported: TGFβ and IL-10 high gene expression was higher in patients with more severe anaemia[56]. Moreover, high gene expression of TNF and TGFβ was more frequent in multitreated patients, suggesting a genetic predisposition to a stronger inflammatory response and a greater immune dysregulation in relapsed or refractory disease[61]. Elevated BAFF cytokine concentrations were also observed after anti-CD20 treatment, which

Box 2 | Childhood AIHA

Epidemiology

Estimated incidence, 0.81 per 100,000 persons per year (95% CI 0.76–0.92)[153]
Mean age at onset, 3 years[154]
Male-to-female ratio, 55% male[154]
Secondary autoimmune haemolytic anaemias (AIHAs) represent 63% of cases[154]
Between 2010 and 2022, the median age of patients with paroxysmal cold haemoglobinuria was 5 years, with 57% of cases occurring in patients aged <10 years[82]

Pathophysiology compared with adult AIHA

Stronger genetic predisposition with underlying inborn errors of immunity (common variable immunodeficiency, autoimmune lymphoproliferative syndrome and others) compared with adults[a]
Consanguinity observed in 8%
Associated immune diseases in 14% of first-degree patient relatives[153]

Distribution of AIHA subtype

Warm AIHAs with IgG only or IgG plus C3d direct antiglobulin test represent 75% of all cases; 23% with C3d only positive direct antiglobulin test, 1% with IgA[153]
Other causes include cold agglutinin syndrome due to infections and, more rarely, paroxysmal cold haemoglobinuria[82]

Treatment and outcome

Corticosteroids are the most common first-line treatment[1,153]
Childhood AIHA requires second-line immunosuppressive therapy in 30–50% of cases[154]
Rituximab and/or immunosuppressors (azathioprine, mycophenolate mofetil, cyclosporin) are the preferred second-line treatments[155]
Antimicrobials may be used in some infection-induced AIHAs
Splenectomy must be avoided in children <5–7 years of age[2]
Overall mortality of childhood primary warm AIHA is 1–2%, higher in cases of Evans syndrome[154]

[a]AIHA in young adults (and rarely also adults) may also reveal a late-onset inborn error of immunity.

Box 3 | Drug-induced immune haemolytic anaemias

Estimated incidence rate
~1 per million persons per year[29]
0.25–0.5% with checkpoint inhibitors (more frequent with anti-PD1, such as nivolumab, than with anti-PDL1 or anti-CTLA4)[30,31]

Main mechanisms[29]
Drug-dependent immune haemolytic anaemia is the most common form of drug-induced immune haemolytic anaemia (DIIHA) in which antibodies only react in vitro in the presence of the drug (most DIIHAs are induced by antibiotics). Many drugs can attach to red blood cells (RBCs), leading to antibodies directed to the drug, drug–membrane proteins and/or membrane proteins. In addition, some antibodies may also be directed at metabolites of the drug and may or not react with the parent drug[156].
In drug-independent immune haemolytic anaemia, the causative drug (for example, α-methyldopa) induces an autoimmune reaction towards self-RBCs, leading to the production of authentic anti-erythrocyte antibodies, some of which can trigger autoimmune haemolytic anaemia[156].
Complement-mediated haemolysis is most often involved in DIIHA, leading to acute and sometimes severe intravascular haemolysis[29,156].

Temporal association for causative drug identification
Temporal association is not a strong indicator of which drug has caused the haemolysis as this may occur from days to weeks or even months following the introduction of a suspected drug[29]. The role of concomitant medications is also hard to assess[156].
Resolution of haemolysis after removal of the suspected drug provides stronger evidence.

Diagnosis[153]
A positive direct antiglobulin test with monospecific antisera (anti-IgG and anti-C3) confirms the immune mechanism of haemolytic anaemia (5–10% of DIIHAs are negative in the direct antiglobulin test)[29,156].
DIIHA caused by drug-independant antibodies are indistinguishable from autoimmune haemolytic anaemia as drug-independent antibodies cannot be ascertained or excluded by serological testing.
DIIHA caused by drug-dependent antibodies can be confirmed by a reference laboratory by obtaining an eluate of patient RBCs and testing the eluate in the presence of RBCs pre-treated with the suspected drug (hapten inhibition for confirmation) or its metabolites[85].

Drugs most frequently[a] involved in DIIHA (by specialty)[30,85,157]
Cardiology: α-methyldopa, furosemide, hydrochlorothiazide.
Anti-infective agents: ceftriaxone, cefixime, piperacillin, amoxicillin with or without clavulanic acid, sulfamethoxazole, trimethoprim, ciprofloxacin, norfloxacin, amphotericin B.
Oncology: carboplatin, oxaliplatin, checkpoint inhibitors[b] (nivolumab, pembrolizumab, ipilimumab, atezolizumab), fludarabine.
Rheumatology: NSAIDs (ibuprofen, diclofenac), allopurinol.
Neurology: apomorphine, levodopa (in association).
Various: acetaminophen, azathioprine, iomeprol.

Management
Management is mainly based on interruption of the suspected drug[85,157].
For most DIIHAs:
Improvement or recovery of haemolytic anaemia is expected 1–2 weeks after drug discontinuation[29,156].
Supportive measures with transfusion of packed RBCs if needed.
For DIIHA induced by checkpoint inhibitors[31]:
Corticosteroids, rituximab may be considered for corticosteroid-resistant cases.
Re-challenge with the same drug can be discussed based on assessement of individual benefit-to-risk ratio. In case of re-challenge, relapse occurs in ~50% of cases.

[a]This is a non-exhaustive list of drugs causing DIIHA. [b]An underlying lymphoproliferative disorder is found in ~40% of cases of checkpoint inhibitor-induced autoimmune haemolytic anaemias.

contributed to the emergence of a population of autoreactive splenic long-lived plasma cells, explaining the absence of response after B cell depletion in some patients and the perpetuation of the autoimmune process[49]. In addition to the anti-RBC humoral response, cytotoxicity and haemolysis mediated by natural killer cells[62] and CD8 T cell clonal expansions[63] have also been described.

CAD and CAS, origin and properties of cold agglutinins and mechanism of haemolysis

Cold agglutinins are autoantibodies able to bind and agglutinate RBCs at low temperatures with an optimum temperature of 4 °C (Fig. 2). The thermal amplitude of the cold agglutinins, that is, the highest temperature range at which RBC agglutination is induced, is considered as the main determinant of cold agglutinin pathogenicity rather than cold agglutinin titre. Indeed, cold agglutinins with high (>28 °C) thermal amplitudes can induce haemolysis even at mild ambient temperatures[64]. Diseases mediated by cold agglutinins include the classical or primary form of CAD, CAS encompassing some 'secondary' forms of CAD due to well-defined B cell lymphoproliferations such as marginal-zone lymphomas, and cAIHAs induced by infections such as *M. pneumoniae* or Epstein–Barr virus[65].

Autoimmune response. It is well established that CAD corresponds, in ~90% of cases, to a monoclonal gammopathy of clinical significance with IgMκ secreted by an autonomous B cell clone, which behave as an autoantibody targeting the I/i antigens expressed on autologous RBCs. Accordingly, a limited (<10%) nodular bone marrow lymphocytic infiltrate is usually observed in CAD, termed 'CAD-associated lymphoproliferative disorder'[66]. Several cytogenetic abnormalities promoting a putative survival advantage have been described in CAD clones such as trisomy 3, with additional trisomy 12 or 18, and mutations in *KMT2D* and *CARD1* (refs. 65,67,68). The distinguishing features between CAD-associated lymphoproliferative disorder and Waldenstrom macroglobulinaemia are summarized in Table 3.

RBC agglutination is favoured by the pentameric structure of IgM and is responsible for cold-induced ischaemic microcirculatory

symptoms more frequently observed in patients with CAD than in those with CAS[5]. Interestingly, the CAD clones have a restricted immunoglobulin V gene usage, with up to 85% of heavy chains being encoded by *IGHV4-34*, and 59% of light chain genes by *IGKV3-20* (refs. 65,69,70). The *IGHV4-34* gene is characterized by an intrinsic polyreactivity, including reactivity to I/i blood group and providing the basis for RBC recognition by cold agglutinin. This reactivity is mediated by a hydrophobic patch within the framework 1 region of the gene[69]. While this intrinsic self-recognition may seem paradoxical, *IGHV4-34* has been shown to be involved in exogenous antigen recognition after the acquisition of somatic mutations led to a 'redemption' of the autoreactive properties conferred by the hydrophobic patch[45,71].

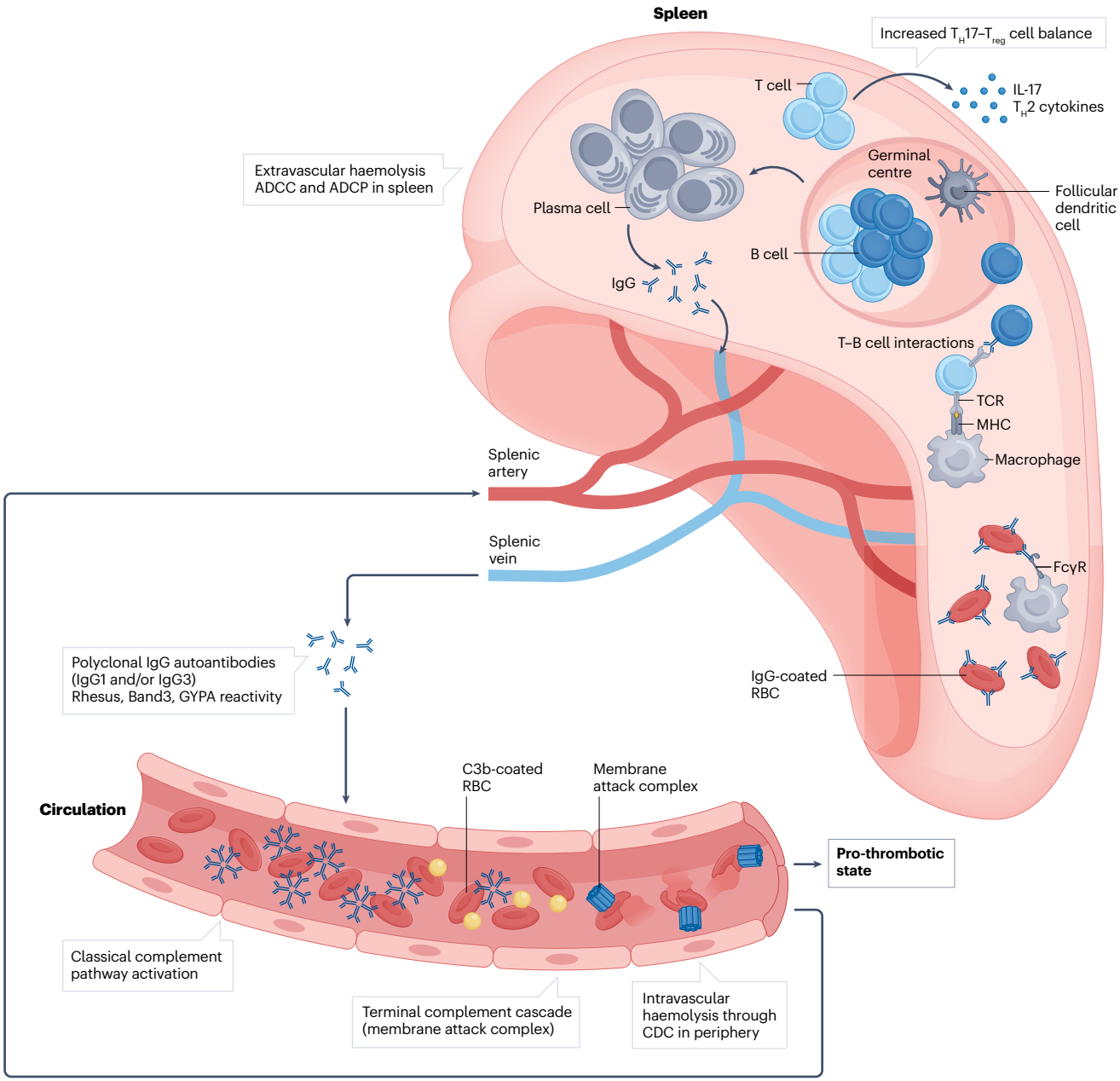


Fig. 1 | Pathophysiology of warm adult autoimmune haemolytic anaemias. The increase of the T helper 17 (T_H17) to regulatory T (T_{reg}) cell balance in the peripheral blood and splenic niche promotes the emergence of autoreactive B cells, leading to the expansion of autoreactive plasmocytes producing polyclonal anti-red blood cell (RBC) IgG antibodies directed towards Rhesus, protein band 3 (Band3) and/or glycophorin A (GYPA) antigens expressed on the surface of RBCs. Within the spleen, IgG-coated RBCs link to Fcγ receptors (FcγR) expressed at the surface of splenic macrophages, leading to their accelerated destruction and extravascular haemolysis through antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC). In parallel, IgG1 and IgG3 autoantibodies activate the classical component pathway and ultimately the terminal complement cascade, leading to various degrees of intravascular haemolysis through complement-directed cytotoxicity (CDC). MHC, major histocompatibility complex; TCR, T cell receptor.

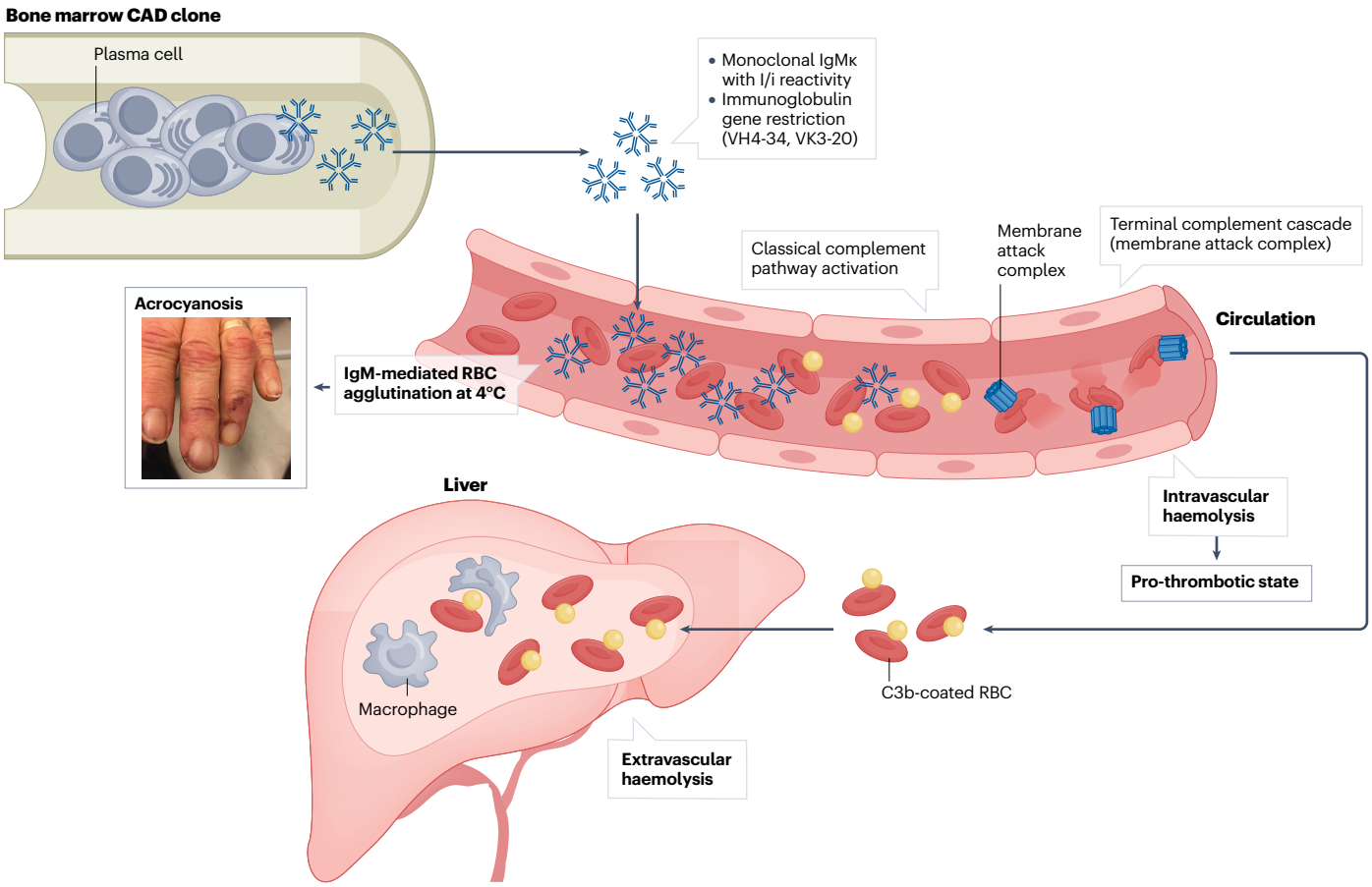


Fig. 2 | Pathophysiology of CAD. A monoclonal IgMκ with anti-I/i autoantibody and cold agglutinin properties originating from a B cell clone in the bone marrow is present in the vasculature. Binding of IgMκ to the erythrocyte surface results in agglutination of red blood cells (RBCs) at cool temperatures, favouring the onset of acrocyanosis. The clonal IgMκ induces complement-dependent haemolysis by activating the classical complement pathway. Extravascular haemolysis of C3b-coated RBCs takes place mostly in the liver but, to a lesser extent, terminal complement activation may also occur in some patients. CAD, cold agglutinin disease.

In CAS secondary to infections, cold agglutinins are polyclonal IgM (rarely IgG) with anti-I/i specificities and usually self-remitting[72]. It has also been suggested that the interaction between *M. pneumoniae* and the I/i antigen on the RBC surface can trigger the autoimmune response[73].

Mechanism of haemolysis. In CAD, the binding of the monoclonal IgMκ to RBCs in the cooler part of the circulation, such as the extremities, leads to the recruitment of C1 on the surface of RBCs and activation of the complement classical pathway. When IgMκ detaches from RBCs in warmer areas of the circulation, C1 remains on their surface. C1 esterase activates C4 and C2, generating a C3 convertase that results in C3 cleavage in C3a (soluble anaphylotoxin) and membrane-bound C3b[65]. This latter component mediates RBC opsonization and phagocytosis by the reticuloendothelial system in the liver[65,74]. To a lesser extent, complement activation on RBCs and subsequent CDC may also be observed in wAIHA but IgM remains the greatest complement activator, followed by IgG3, IgG1 and IgG2, while IgG4 does not activate complement[75]. Although some regulatory proteins mediate complement inhibition on the surface of RBCs, the terminal complement cascade can be also activated in patients with severe disease or acute worsening in both wAIHA and CAD, resulting in intravascular haemolysis[65]. The binding of C4bC2a to C3b generates a C5 convertase, which mediates C5 cleavage in C5a (soluble anaphylotoxin) and membrane-bound C5b that will, in turn, bind C6, C7, C8, and C9 and form the membrane attack complex[76]. Intravascular haemolysis releases erythrocyte microparticles and free haemoglobin, which binds nitric oxide, promoting a pro-thrombotic environment[77]. Indeed, thrombosis has been found to correlate with intravascular haemolysis, and some treatments as well as splenectomy increase this risk[77,78]. Lastly, episodes of infections that give rise to complement activation may classically trigger relapses and/or worsening of haemolysis in patients with CAD.

Diagnosis, screening and prevention

Revealing manifestations and biological features of haemolytic anaemia

Diagnosis of AIHA should be suspected in the presence of unspecific signs and symptoms of anaemia and haemolysis, which may greatly vary depending on the acute or chronic setting, presence or not of

underlying disease or comorbidities, type of AIHA, and site or degree of possible thrombotic-revealing manifestations. Chronic extravascular haemolytic (EVH) anaemia is marked by fatigue, pallor, jaundice, gallstones, hepatomegaly and/or splenomegaly, and iron overload, while acute intravascular haemolysis (IVH) is characterized by additional dyspnoea, tachycardia, fever, dark urine and, sometimes, angina with or without heart failure. Haemoglobin (Hb) values may be highly variable, from <3 g/dl to normal values (12–16 g/dl) if highly effective bone marrow compensation occurs, as expressed by an increased number of reticulocytes (which is inversely related to Hb/haematocrit values). Of note, reticulocytosis may be absent in ~20% of adults and this absence is often associated with increased severity[32]. The definition of severe AIHA is not clearly established but a Hb level of <8 g/dl at onset with transfusion needed at an interval of ≤7 days represents an acceptable definition, although it does not consider tolerance of anaemic state and comorbidities[79]. In the largest retrospective study, including 378 patients with primary AIHA with a median follow-up of 4.3 years, the median Hb at onset was of ~7–7.5 g/dl, with 63% haveing Hb levels of <8 g/dl and 27% of <6 g/dl; 54% required blood transfusions and 64% required hospital admission[32]. In CAD, Hb at onset is higher (9.3 g/dl with 27% <8 g/dl, assessed in 232 patients with a median follow-up of 6 years) but transfusion requirement is comparable with other AIHA subtypes (47% of patients)[9].

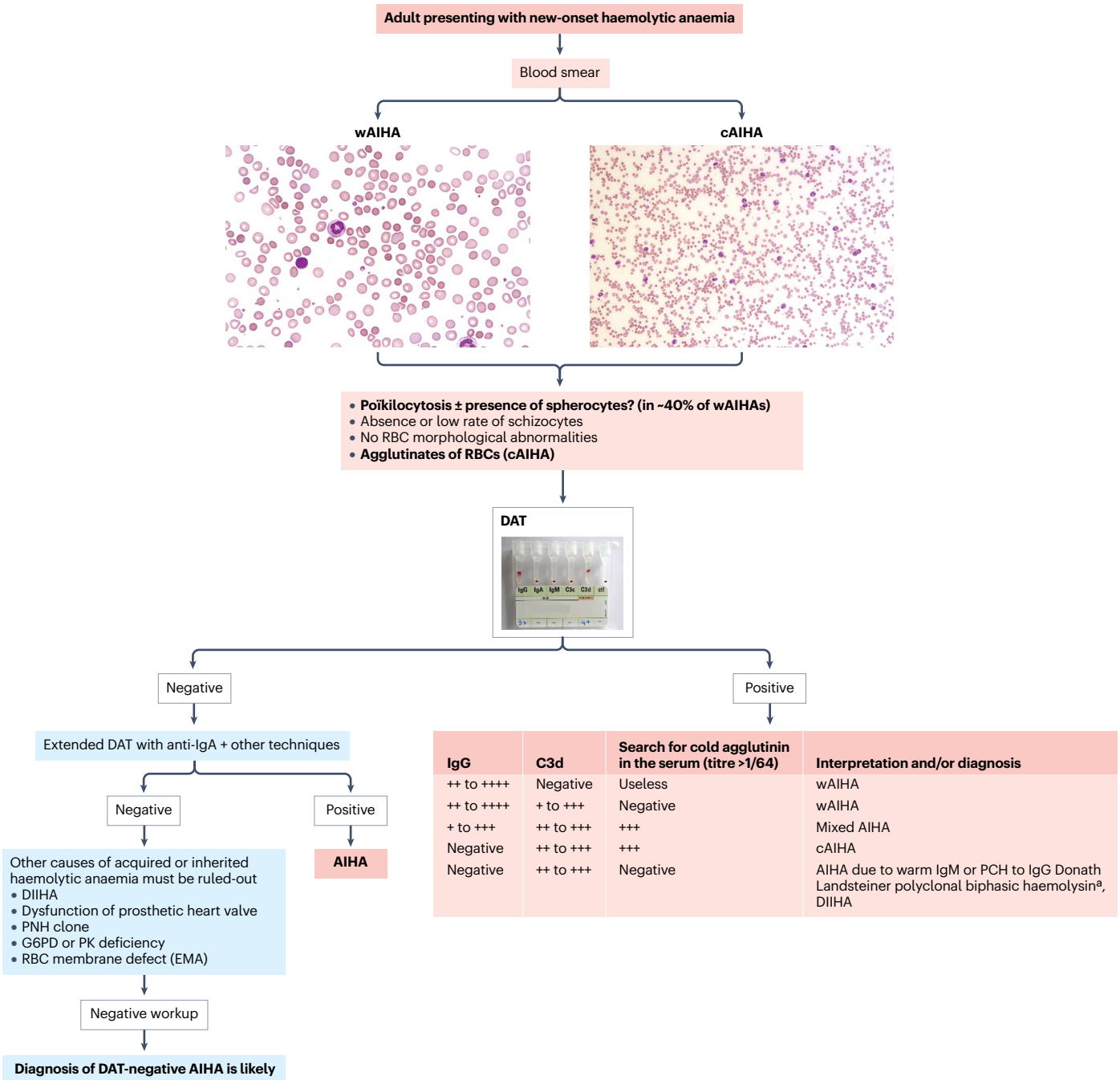
Symptoms of anaemia are associated with Hb values, although equally important is the rapid worsening of anaemia with haemodynamic instability. Unconjugated hyperbilirubinaemia is more typical of EVH occurring in spleen, liver and lymphoid organs, while highly increased LDH (that is, LDH >500 UI/l), plasma-free Hb, haemoglobinuria and haemosiderinuria are hallmarks of IVH occurring in the bloodstream[1,3]. EVH is predominant in classic wAIHA and chronic CAD, whereas IVH is more commonly observed in wAIHA when there is an activation on the classical complement pathway (that is, in ~50% of wAIHAs), during acute crisis of CAD (triggered by cold exposure, infections, surgery, major trauma, stress), and in mixed forms and it is also typical in DIIHA and paroxysmal cold haemoglobinuria[5,80,81]. Circulatory symptoms, such as acrocyanosis defined by a blue colouration of the skin at the extremities, Raynaud phenomenon, ulcerations or, exceptionally, gangrene, in cold-exposed areas of the body are typical of CAD as well as spontaneous agglutination of blood samples at room temperature, which may generate an alarm from the laboratory because of unreliable blood counts[65]. Finally, haptoglobin is almost invariably consumed, being one of the most sensitive haemolytic markers, which may be reduced even after complete recovery[82]. The initial clinical picture may be confused by underlying or associated conditions, present in ~50% of patients with wAIHA (Table 2). Once the haemolytic mechanism is recognized, the first step of the diagnostic procedure is a careful analysis of the peripheral blood smear, which is crucial for ruling out other diagnoses such as microangiopathy or morphological abnormalities of RBCs (such as sickle cells) suggesting an underlying, previously unrecognized inherited cause of haemolytic anaemia. In wAIHA, the presence of spherocytes, resulting from an incomplete phagocytosis of opsonized RBCs, is found in ~40% of cases[24], whereas by definition, the presence of RBCs agglutinates is the hallmark of CAD and CAS.

Diagnosis of AIHA

Confirmation of the immune mechanism of haemolysis relies on the DAT, which is the cornerstone in the diagnosis of AIHA[1,5] (Fig. 3). The DAT is based on the addition of an antiglobulin reagent that reacts with the autoantibody bound to patient erythrocytes, promoting their agglutination in vitro. It is commonly performed with polyspecific antisera and results in a semi-quantitative positivity (generally expressed as 1+ to 4+ or more), without distinguishing the isotype of the autoantibody[5]. Therefore, it is currently mandatory to perform the DAT with monospecific antisera, namely anti-IgG, and anti-complement (C3d), to properly distinguish the different forms of AIHA, as therapy for each is different. When the DAT is negative, a complementary study with anti-IgM and anti-IgA monospecific antisera in a reference laboratory must be performed. Notably, IgM are potent activators of the classical complement pathway but may detach from the RBCs during washing procedures or even eluted in vivo, thus giving a negative result. In this case, however, the DAT remains strongly positive for C3d, which can be considered a surrogate of IgM positivity. Additionally, some IgG subclasses (IgG3, IgG1 and, to a small extent, IgG2) are also able to activate complement,

Table 3 | Comparisons of the characteristics of cold agglutinin disease-associated lymphoproliferative disease and Waldenstrom macroglobulinaemia/lymphoplasmocytic lymphoma

	Cold agglutinin disease	Waldenstrom macroglobulinaemia
Monoclonal component	Low intensity (<10 g/l) IgM (90% of cases) No risk of hyperviscosity syndrome	IgM of variable intensity, can be >30 g/l Induced hyperviscosity syndrome
Light and heavy chain restriction	IgH24-34 (>85%) IgKV3-20 (59%)	IgHV3 (83%), IgHV3-23 (24%)
Enlarged lymph nodes±spleen at disease onset	None in the majority of cases	Present in 20–25% of cases
Common features on bone marrow biopsy	Nodular infiltrate (≤10%) of small B cells; $CD19^+$, $CD20^+$, $CD22^+$, $Bcl6^-$, $CD27^+$, $CD79^+$, $FMC7^+$, $IgM\kappa^+$, IgD^+, $CD5^{\pm}$, $CD23^-$, $CD38^-$ Absence of lymphoplasmocytes, weak (<5%) extranodular infiltrate of plasmocytes, no fibrosis, no paratrabecular infiltrate	Lymphoplasmacytic interstitial infiltrate (>10%) $CD20^+$ $CD138^+$ and paratrabecular $CD19^+$, $CD20^+$, $CD22^+$, $CD25^+$, $CD27^+$, $FMC7^+$, IgM^+, $CD5^{\pm}$, $CD23^-$, $CD10^-$
Type of somatic mutations	*KMT2D* (*MLL2*) and *CARD11* mutations (30%) *MYD88* L2659 (0–20%)	*MYD88* L2659 (>90%) *CXCR4* (30–35%)
Cytogenetics	No specific associated cytogenetic abnormalities	$6q^-$ (30–50%)
Risk of transformation into an aggressive B cell lymphoma	Rare (3.5%)	Possible (5–15%)


Adult presenting with new-onset haemolytic anaemia
Blood smear
wAIHA
cAIHA
• Poïkilocytosis ± presence of spherocytes? (in ~40% of wAIHAs)
• Absence or low rate of schizocytes
• No RBC morphological abnormalities
• Agglutinates of RBCs (cAIHA)
DAT
IgG IgA IgM C3c C3d ctl
Negative
Positive
Extended DAT with anti-IgA + other techniques
Negative
Positive
AIHA
Other causes of acquired or inherited haemolytic anaemia must be ruled-out
• DIIHA
• Dysfunction of prosthetic heart valve
• PNH clone
• G6PD or PK deficiency
• RBC membrane defect (EMA)
Negative workup
Diagnosis of DAT-negative AIHA is likely

IgG	C3d	Search for cold agglutinin in the serum (titre >1/64)	Interpretation and/or diagnosis
++ to ++++	Negative	Useless	wAIHA
++ to ++++	+ to +++	Negative	wAIHA
+ to +++	++ to +++	+++	Mixed AIHA
Negative	++ to +++	+++	cAIHA
Negative	++ to +++	Negative	AIHA due to warm IgM or PCH to IgG Donath Landsteiner polyclonal biphasic haemolysin[a], DIIHA

Fig. 3 | Diagnostic procedure for AIHA. When diagnosing a patient with a new-onset haemolytic anaemia and autoimmune haemolytic anaemia (AIHA) is suspected, the diagnosis of AIHA relies on a multiple-step procedure, including first the analysis of the peripheral blood smear. When the blood smear shows no abnormalities or the presence of spherocytes (~40% of warm AIHAs (wAIHAs)) or spontaneous red blood cell (RBC) agglutinates (cold agglutinin disease or cold agglutinin syndrome, drug-induced immune haemolytic anaemia (DIIHA)), the next step is the direct antiglobulin test (DAT). A positive DAT confirms the diagnosis of AIHA and, in combination with the presence of cold agglutinin in the serum, a diagnosis of AIHA can be made. When DAT is negative, other causes of inherited and acquired haemolytic anaemias must be ruled out before considering the diagnosis of DAT-negative AIHA (~5% of all AIHAs). cAIHA, cold AIHA; EMA, extracellular membrane of band 3 protein by eosin-5′-maleimide; G6PD, glucose 6-phosphate dehydrogenase; PCH, paroxysmal cold haemoglobinuria; PK, pyruvate kinase; PNH, paroxysmal nocturnal haemoglobinuria. [a]Flow cytometry solid-phase, low ionic strength or cold solutions for washing, cytometry, ELISA, Dual-DAT, mitogen-stimulated DAT.

resulting in a weak anti-C3d positivity. Finally, autoantibodies of the IgM class are pentamers, able to cause direct agglutination of erythrocytes at 20 °C, a very simple and old test[25].

Problems with DAT and alternative tests. The DAT reports false negatives in ~5–10% of AIHAs due to several reasons. Firstly, the content of anti-IgG, anti-IgM, anti-IgA and anti-complement fractions in polyspecific antisera is highly variable, and as they contain mostly anti-IgG, they might give false negative results in cases where there are autoantibodies of other specificities (that is, IgM or IgA). The use of monospecific antisera may overcome this problem (particularly for IgA autoantibodies). The second reason is that autoantibodies may have low affinity and may thus detach from erythrocytes during washing procedures. This problem may be solved by using low ionic strength or cold solutions. Lastly, the test has low sensitivity, for example, the classic tube requires ~400 molecules of autoantibody to give a positive result; therefore, it is currently replaced or supported by semi-automated methods such as the gel microcolumn[1] (Fig. 3) and solid-phase tests, with better sensitivity and acceptable specificity. Sensitivity may also be highly improved by experimental techniques like ELISA, flow cytometry (able to detect ~40 molecules of autoantibody) and mitogen-stimulated DAT, which increase the amount of RBC-bound IgG and could disclose a latent autoimmunity[25]; however, their positivity should be cautiously interpreted in the clinical context given their lower specificity. Additional tests in reference laboratories include the dual-DAT[83], which is useful for the detection of IgM warm antibodies often responsible for severe and fatal AIHA[35]. Finally, Donath–Landsteiner biphasic haemolysin (an antibody that binds in cold temperatures and haemolyzes at body temperatures) is the cornerstone of the diagnosis of paroxysmal cold haemoglobinuria[81], a rare form of AIHA occurring almost exclusively in children.

A false positive DAT may also occur in various circumstances, including the adsorption of cytophilic antibodies (that is, antiphospholipid antibodies), the increasing use of certain drugs (that is, the anti-CD38 daratumumab), the presence of alloantibodies due to prior transfusion, and the use of high-dose intravenous immunoglobulin[84]. The indirect antiglobulin test reveals the presence of autoantibodies or alloantibodies in patient serum by its incubation with standardized RBC panels of known antigenicity. It is not strictly necessary for the diagnosis of AIHA but it is very helpful in assessing the presence of underlying alloantibodies, which can often accompany autoantibodies. The indirect antiglobulin test may also enable the characterization of antigen specificity, generally the Rhesus system in warm forms and I/i antigens in CAD. Serological studies are important in CAD to determine the titre of cold agglutinins; generally, values greater than 1/64 are required to confirm the diagnosis of CAD, while lower titres (<1/64) are associated with secondary forms[5]. Finally, serological tests may include the study of the eluate, that is, the antibodies eluted from autologous erythrocytes, which are particularly useful in difficult cases and in the diagnosis of drug-induced AIHAs[85].

Extra testing for confirmation of AIHAs

Several other factors should be considered in evaluating anaemia and haemolytic markers with a particular focus on bone marrow evaluation, which is not routinely performed in AIHAs (Box 4). Bone marrow morphology, cytometry, cytogenetics and biopsy may be advised in wAIHAs with reticulocytopenia as well as in wAIHAs refractory to first-line therapy with steroids and in CAD at diagnosis along with a whole-body CT scan to diagnose secondary forms[5,85]. Other reasons for considering the analysis of bone marrow is the possible evolution of AIHAs into myelodysplasia or bone marrow failure syndromes (either spontaneous or therapy induced) or to evaluate the type of lymphocyte infiltrate (T or B) that may aid in the choice of appropriate targeted therapy. In CAD requiring specific therapy, bone marrow evaluation is strongly recommended since the disease is usually accompanied by a clonal, low-grade lymphoproliferation without *MYD88* L265P mutation (Table 3), currently included in the World Health Organization classification of haematolymphoid tumours. Box 4 reports the several investigations proposed in AIHAs, including screening for autoimmunity and immunodeficiency[2,3].

In summary, the DAT is fundamental for the diagnosis of AIHAs although it must be cautiously interpreted as it is neither 100% sensitive nor specific. Likewise, anaemia and alteration of haemolytic markers have several confounding factors and underlying conditions may complicate the clinical picture. A careful medical history taking and several investigations are required for a proper diagnosis along with continuous dialogue with a reference laboratory with a comprehensive diagnostic package.

Screening and prevention

There is only little room for screening in the setting of AIHAs since there is no strong genetic predisposition; additionally, for patients at risk of developing secondary AIHA, the onset of AIHA is most often unpredictable. For patients diagnosed with CLL, a DAT should be systematically performed as patients with a positive DAT at the time of CLL diagnosis are more prone to developing AIHA during follow-up and should not be treated with fludarabine, which may trigger AIHA[1]. More generally, for patients diagnosed with a disease predisposing to secondary AIHA, such as SLE, beyond the positivity of the DAT and/or a history of immune thrombocytopenia, there is no reliable predictor of AIHA.

Management

The management of AIHAs includes some preventive and supportive measures that must not be neglected as well as more specific therapies, which may differ according to the type of AIHA.

Prevention of AIHA relapse or worsening and thrombosis

The prevention of common community-acquired infections that may activate the complement cascade and trigger AIHA relapse or worsening is strongly recommended. Therefore, vaccinations against *Streptococcus pneumoniae*, flu and SARS-CoV-2 must be considered in patients with AIHA. Those with CAD should also be vaccinated against different serotypes of *Neisseria meningitidis* before being treated with complement inhibitors such as sutimlimab[86].

Patients diagnosed with CAD or CAS must avoid, as much as possible, the exposure to cold (that is, less than ~10 °C) temperatures as well as sudden temperature variations to limit the risk of severe microcirculatory manifestations and, to a lesser extent, exacerbation of haemolysis[9]. Moreover, in case of a planned surgical procedure and especially cardiac surgery requiring extracorporeal circulation, the anaesthesiologist must be aware of the risk of acute and potentially life-threatening haemolysis and/or massive agglutination of RBCs, and take the appropriate measures[87].

Since erythropoiesis is increased in AIHAs, a daily oral supplementation with folic acid to prevent the occurrence of vitamin B9 deficiency over time is common practice[1]. Patients with AIHAs have an increased risk of thromboembolic events, particularly deep vein thrombosis and/or pulmonary embolism[7,88]; therefore, preventive anticoagulation should be considered for every patient

Box 4 | Recommended work-up for a suspicion of AIHA

Diagnosing autoimmune haemolytic anaemia (AIHA)

Complete blood count plus reticulocyte count and standard haemolytic markers (unconjugated bilirubin, LDH, haptoglobin), renal and liver functional tests

To confirm the haemolytic mechanism of anaemia with compensatory increased erythropoiesis (reticulocytosis) as well possible consequences of massive intravascular haemolysis such as renal insufficiency. Mean corpuscular volume is most often falsely increased because of reticulocytosis.

Consider confounding factors:

(1) Unconjugated hyperbilirubinaemia is also present in Gilbert syndrome.

(2) Increased LDH accompanies several diseases with cellular necrosis or increased tissue turnover (for example, myocardial infarction, myositis, solid and haematological tumours) and vitamin B12 deficiency.

(3) Reduced haptoglobin is also present in congenital hypohaptoglobinaemia, severe liver impairment and malnutrition; conversely, it may be falsely normal or increased in inflammatory diseases, cigarette smokers and nephritic syndrome possibly masking haemolysis.

(4) Reticulocytosis is also observed in pregnancy or delivery, acclimation, and increased erythropoiesis after an episode of bleeding (which may also worsen haemoglobin values); conversely, normal reticulocyte count, or even more rarely reticulocytopenia, may be seen observed in up to 20% of AIHAs, possibly due to antibodies against erythroblasts that impair bone marrow compensation or in case of concomitant vitamin B9 or B12 deficiency.

Blood smear examination

May help in differential diagnosis of haemoglobinopathies and congenital haemolytic anaemias as well as microangiopathy (Fig. 2).

Direct antiglobulin test with monospecific antisera

To confirm the immune origin of haemolytic anaemia and distinguish between warm, cold or mixed AIHAs (Fig. 2).

Consider false positive and negative test (perform more sensitive tests in a reference laboratory in case of persistent clinical suspicion).

Cold agglutinin titre

When direct antiglobulin test is positive for C3d.

Diagnosing primary or secondary AIHA

Autoimmune screening

Anti-nuclear antibodies, anti-DNA, anti-extractable nuclear antigens (anti-ENA) ± lupus anticoagulant, anti-cardiolipin and anti-β2 glycoprotein 1 antibodies if anti-nuclear antibodies are positive and/or previous history of thrombosis.

To search for an associated underlying systemic autoimmune disease.

Consider infectious disease screening based on the context

SARS-CoV-2, parvovirus B19, *Mycoplasma pneumoniae*, Ebstein–Barr virus, cytomegalovirus, hepatitis C virus, HIV.

In case of infectious prodromal and/or clinical suspicion of an infectious trigger.

Chest and abdominal CT scan

To look for an underlying thymoma, solid tumour in the presence of warm AIHA or features of lymphoma in warm AIHAs, mixed AIHAs or cold agglutinin syndrome without any infectious trigger. Of note, mild splenomegaly due to extravascular haemolysis is common in active warm AIHA.

Serum protein electrophoresis and immunofixation

Serum IgG, IgA and IgM levels, B lymphocyte subpopulations by cytometry.

To search for an underlying immunodeficiency such as common variable immunodeficiency, a monoclonal gammopathy or for some evidence of an underlying B cell lymphoproliferation (chronic lymphocytic leukaemia, marginal-cell lymphoma).

Bone marrow evaluation (morphology, cytometry, cytogenetics and/or biopsy)

May be useful in refractory warm AIHAs or in case of reticulocytopenia. It is recommended in cold agglutinin disease at diagnosis, to rule out secondary causes (chronic lymphocytic leukaemia, lymphoma). It is important to evaluate inadequate compensation (concomitant myelodysplasia) and useful to assess the possible evolution of AIHAs to myelodysplasia or bone marrow failure syndromes.

during disease worsening or relapse requiring hospitalization. Daily administration of low-molecular-weight heparin is recommended[1,88]. Whether patients with a previous history of thrombosis who are not on continuous anticoagulation and/or those with other inherited or acquired thrombophilic risk factors could benefit from higher doses of low-molecular-weight heparin or even full-dose anticoagulation is not established.

Supportive measures

Transfusion. Blood transfusions for patients with AIHA are challenging. First, providing patients with serologically compatible RBCs may be difficult since the autoantibodies can mask the presence of alloantibodies and may react to all screening cells and donor cells[89]. Secondly, there is an increased risk of haemolytic transfusion reactions in this setting and alloantibodies can be found in up to 40% of patients with AIHA[89]. However, when the correct work-up and compatibility testing procedures for appropriate matching of patients and donors in order to select the optimal RBC units[89–92] are carefully made by the blood centre, the risk of haemolytic transfusion reactions and exacerbation of haemolysis is actually very low and the immediate efficacy of transfusion is good in at least two-thirds the patients[91–94]. Taking this into consideration, transfusion of packed RBCs is most often indicated and should not be postponed in patients aged >70 years with comorbidities and an Hb level <8 g/dl or in younger patients with more severe anaemia (that is, Hb ≤6 g/dl) for whom the short-term mortality remains relatively high.

Recombinant erythropoietin. The transient off-label use of recombinant erythropoietin (Epo) may be helpful as a transfusion-sparing

strategy for patients with features of inadequate erythropoiesis assessed by a low bone marrow responsiveness index (calculated as absolute reticulocyte count × Hb level/normal Hb level/1,000) <121)[32,95] with or without an inappropriate level of endogenous Epo[95]. Two retrospective studies[96,97] and one prospective study on 47 patients[95] have shown that the use of Epo in wAIHAs or cAIHAs may increase reticulocyte count and, ultimately, the Hb level in up to 70–75% of patients with inadequate bone marrow compensation. Either darbopoietin-α or epoietin-α can be used in this setting. Based on clinical experience and the data from the literature, the use of Epo in the context of AIHAs does not seem to increase the risk of thromboembolic events[95,96].

Rescue therapies

Intravenous immunoglobulin (IVIg) is sometimes used off-label in severe AIHAs requiring repeated transfusions but their expected efficacy is low and only minimal data are available in the literature. In the sole large series reported so far on 37 patients, including 5 children, the ORR was 32.4%[98], and in a recent French multicentre observational study on 34 adult patients with severe and/or transfusion-dependent AIHA treated with IVIg, the ORR was 32.4% on day 7 (ref. 99). Based on these data and the absence of clear predictors of response, the use of IVIg should be considered only for patients with life-threatening AIHAs needing repeated transfusion who do not respond rapidly to first-line treatment. As seen in other autoantibody-mediated autoimmune diseases, plasma exchange (PEX) may also be used occasionally for managing severe AIHAs. However, based on the minimal data available in the literature, the efficacy of PEX is somewhat controversial[100] and difficult to assess as patients with life-threatening AIHA usually receive many concomitant therapies[101]. Therefore, as for IVIg, the use of PEX as a rescue treatment of severe AIHA should be limited and discussed with a reference centre and compensation should rely on albumin rather than on fresh frozen plasma, which may further activate complement. Of note, the effectiveness of PEX may depend, at least in part, on the isotype of the autoantibody. IgM antibodies are removed much more effectively than IgG because a significantly greater proportion are limited to the intravascular space. This is reflected by the guidelines produced by the American Society for Apheresis, wherein severe CAD is considered a category II indication for PEX while severe wAIHA is a category III indication[102].

Treatment of wAIHAs

Corticosteroids remain the backbone of first-line therapy for wAIHA. Predniso(lo)ne is usually used at a high dose for 2–3 consecutive weeks and then slowly tapered and stopped within 3–6 months in patients who achieve an initial response[1,5]. Similar to other autoimmune diseases, a high dose of methylprednisolone or dexamethasone (DXM) is sometimes used for the most severe cases but there is only minimal evidence suggesting a higher and/or faster efficacy when compared with predniso(lo)ne[103,104]. If, on average, 80% of patients achieve an initial response (that is, increase in Hb level above 10 g/dl with at least a 2 g increase from baseline) with corticosteroids alone[24], based on a retrospective study and on two randomized prospective studies, only about a third of them will achieve a durable response upon discontinuation[24,105,106]. For corticosteroid-resistant or corticosteroid-dependent wAIHAs, rituximab (RTX) has shown good efficacy in two randomized controlled trials, one of which was double blind versus placebo, with an ORR of 75% at 1 year[105,106]; therefore, RTX is considered as the preferred second-line option[1,107]. RTX is usually administered at standard dose[105,106] but lower doses have also been shown to induce a sustained response in wAIHA in a phase II clinical trial[108].

For patients with an abrupt onset and severe anaemia (that is, Hb level <6 g/dl) or in older patients with comorbidities (for example, diabetes, hypertension or obesity), the use of RTX upfront as a first line in combination with prednisone must be considered (Fig. 4). For patients with a history of severe infusion-related reaction to RTX or serum sickness, like in patients with CLL, the use of a fully humanized anti-CD20, such as obinutuzumab, may be efficacious[109]. In the absence of response to RTX or in case of early relapse after an initial response, the use of an immunosuppressor is now usually preferred to splenectomy[1]. Azathioprine, which is the only immunosuppressor licensed for wAIHA, leads to a 50–60% response[1,5] but there is no data available showing its superiority over mycophenolate mofetil or cyclosporin, which are the usual alternatives[110–112]. A patient not responding to both RTX and at least one immunosuppressor should be advised to undergo splenectomy, which was historically the best second-line option. In primary wAIHA, splenectomy leads to a 50–60% durable response rate[113,114] with the main limitations being the increased risk of overwhelming post-splenectomy infections and thromboembolic events. There is no consensus for the best management of patients with refractory wAIHAs. They may either be included in a clinical trial or be treated off-label with the proteasome inhibitor bortezomib combined with DXM in order to target long-lived autoreactive long-lived plasma cells[115,116]; for the latter, promising efficacy was reported in a series of eight patients with refractory wAIHA[117]. Another small prospective study showed a response to a combination of bortezomib, low-dose RTX and DXM in six out of seven patients with refractory wAIHA[117].

For secondary wAIHAs, the underlying disease may influence the therapeutic strategy. In case of active (that is, stage B or C) CLL, for example, the use of a regimen combining RTX with DXM and cyclosphosphamide can be an option[118] as well as a combination of RTX plus bendamustine[119]; other drugs such as ibrutinib or venetoclax may be helpful for managing the more refractory cases[120]. Conversely, RTX alone is usually highly effective in SLE-associated or common variable immunodeficiency-associated wAIHA[121,122], two conditions in which splenectomy should be avoided.

Treatment of CAD

Unlike wAIHAs, corticosteroids at standard dose are of little, if any, efficacy in CAD, with only 15–20% of patients being initial responders[1,5], and should therefore not be used in the long term in CAD or CAS; the same applies to other classical immunosuppressors. Furthermore, considering that, in CAD, the destruction of RBCs mediated by C3b takes place in the liver, splenectomy is not effective nor recommended[1]. Patients with CAD with mild or partially compensated haemolytic anaemia (that is, with Hb level ≥10 g/dl) and no or only moderate microcirculatory symptoms, representing 30–50% of patients, can only be managed with preventive measures[9]. Conversely, for patients with CAD who have active haemolysis with a Hb level that is constantly or regularly below 10 g/dl (regardless the need of regular transfusions) and/or, to a lesser extent, for those with severe microcirculatory symptoms, a specific treatment is indicated.

Two therapeutic strategies can be used for CAD. The first unique option is to target the underlying B cell clone giving rise to the monoclonal IgMκ with autoantibody properties[5]. The second option is to inhibit the activation of the classical complement pathway downstream to switch off the haemolytic process[65]. To target the B cell clone, based on some uncontrolled prospective studies, the off-label use of RTX at

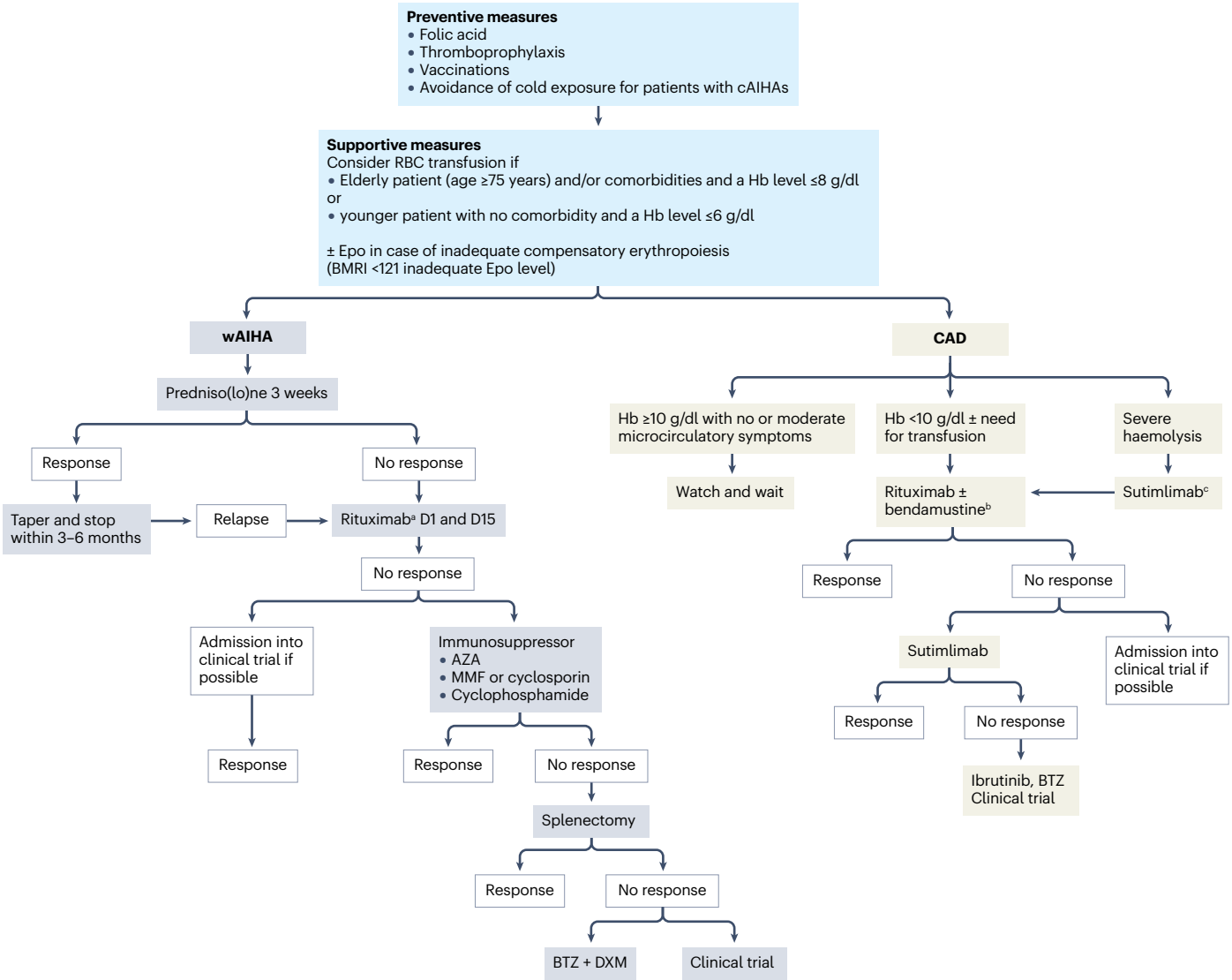


Fig. 4 | Treatment algorithm for primary wAIHA and CAD. The management of autoimmune haemolytic anaemia (AIHA) relies first on preventive and supportive measures, including avoidance of cold exposure for patients with cold agglutinin disease (CAD) or cold agglutinin syndrome and prompt transfusion of red blood cells (RBCs) for patients with profound anaemia and/or older patients with comorbidities and with a haemoglobin (Hb) level <8 g/dl. Subsequent therapy must consider the type of AIHA. Corticosteroids are the cornerstone of treatment for warm AIHAs (wAIHAs) but are of little, if any, efficacy for CAD. Rituximab is the preferred second-line used as a corticosteroid-sparing agent in wAIHAs, whereas up to 50% of patients of CAD can be only watched and managed with supportive and symptomatic measures. For those patients with CAD and a Hb level <10 g/dl, treatment with rituximab ± bendamustine is relevant to target the B cell clone, increases Hb level and potentially also improves cold-induced microcirculatory symptoms. For patients with active haemolytic anaemia who do not respond to rituximab ± bendamustine, the use of sutimlimab can be considered whenever available. AZA, azathioprine; BMRI, bone marrow responsiveness index; BTZ, bortezomib; cAIHAs, cold AIHAs; D, day; DXM, dexamethasone; Epo, erythropoietin; MMF, mycophenolate mofetil. [a]Rituximab can be administered earlier as first line in combination with predniso(lo)ne in elderly patients with comorbidities (that is, diabetes, hypertension, obesity) or in case of severe anaemia requiring repeated transfusion. [b]In fit patients only. [c]Transient use as a bridging therapy upon availability.

standard dose leads to an ORR of 50% at 1 year with minimal toxicity[123]. However, beyond 1 year, most of initial responders relapse[5,123]. Combining RTX with bendamustine increases the ORR up to 75% at 1 year and leads to more complete and durable responses but with a substantial risk of neutropenia and severe infections that may be difficult to overcome for unfit patients and/or those aged >70 years[124]. In case of refractoriness to RTX or early relapse after RTX ± bendamustine, other options for targeting the clone include ibrutinib, which showed a 100% response rate in a small retrospective series including both patients with primary CAD or secondary (that is, CAS)[125]; venetoclax or bortezomib (off-label use)[1,126] may also be considered. Beyond its potential efficacy for controlling haemolytic anaemia by directly targeting the IgM autoantibody, this strategy can also improve associated microcirculatory symptoms.

For the very few CAD patients (~10%) with no detectable clone, for those with severe and sometimes life-threatening haemolysis requiring repeated transfusions, or for patients who fail RTX, the inhibition of complement activation is clearly relevant. Since the haemolytic process is essentially mediated by C3b in the liver and the activation of the terminal complement pathway into the vasculature plays only a minor role, inhibiting C5 with eculizumab is of little efficacy in CAD[127]. However, based on two prospective trials, one of which was controlled and randomized versus placebo, sutimlimab, a C1s inhibitor, has shown a high efficacy and a good safety profile[86,128] and is now licensed for this indication in many countries. Administered intravenously at a fixed dose every 2 weeks, sutimlimab led, irrespective of previous exposure to RTX, to a rapid response in 54–73% of patients, with an average increase in Hb level of 2.6 g/dl and a substantial concomitant improvement of quality of life (QoL), especially the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue score[86,128–131]. The efficacy of sutimlimab persists over time as long as treatment continues but, as expected based on its mechanism of action, a recurrence of haemolytic anaemia occurs within a few weeks after its interruption[86,128]. However, further post-marketing, real-world studies with a long-term follow-up are required prior to drawing final conclusions regarding the place of sutimlimab in the management of CAD.

Riliprubart is a second-generation humanized monoclonal antibody (mAb) that selectively inhibits the activated form of C1s and requires intravenous administration every 12 weeks. In one phase Ib study, a single intravenous dose of riliprubart was well tolerated and led to rapid classical Ct inhibition, control of haemolysis, and improvement in haemolytic anaemia, all of which were sustained over 15 weeks[132]. Other inhibitors of the classical complement pathway are currently under development in this indication (see Outlook section). Of note, based on their mechanism of action, complement inhibitors have no effect on IgM-mediated microcirculatory symptoms[5].

There is no consensus for the treatment of CAS. Secondary forms of CAD due to well-defined B cell lymphomas should be treated with RTX ± chemotherapy or with other regimens according to the standard of care. Sutimlimab, which has not been tested in these indications, could be helpful in the future as a bridging therapy for patients with severe haemolysis. CAS secondary to infections is usually managed only with supportive measures along with treatment of the underlying infection whenever available. There is no evidence showing that a short course of corticosteroids may reduce the duration of haemolytic anaemia and improve the outcome[1,5].

Lasty, treatment of mixed AIHA, an uncommon type of AIHA with a more aggressive course, involves primarily the use of corticosteroids, RTX and splenectomy with only limited evidence available[1,5].

New therapies under development in primary wAIHAs

Ianalumab. Ianalumab (VAY736) is a human IgG1/κ mAb targeting B cell activating factor (BAFF) receptor (BAFFR). BAFF is a cytokine belonging to the TNF superfamily, is involved in B cell survival, maturation and differentiation, and the levels of BAFF have been correlated with the activity of wAIHA[40]. Ianalumab eliminates BAFFR$^+$ mature and immature B cells via a dual mechanism involving ADCC and induction of B cell apoptosis by blocking BAFF–BAFFR interactions and the downstream survival pathway in B cells[133]. There is some evidence showing that the magnitude of B cell depletion induced with ianalumab is higher than that observed with anti-CD20 mAbs such as RTX and obinutuzumab. Based on this rationale, a phase III study is ongoing in wAIHA (Table 4).

Povetacicept. Povetacicept is an engineered double inhibitor of soluble forms of BAFF and APRIL (a proliferation-inducing ligand). These molecules act on B cells through several receptors, transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI), BAFFR and, to a lesser extent, through B cell maturation antigen (BCMA), to induce B cell maturation and survival. APRIL and BAFF play non-redundant roles in B cell regulation, due in part to differential receptor expression at partially overlapping stages of B cell development[134]. A phase Ib open-label study in individuals with autoimmune cytopenias, including wAIHA and CAD, has been initiated (NCT05757570).

Parsaclisib. Parsaclisib is a potent inhibitor of PI3 kinase PI3Kδ, with ~20,000-fold selectivity for PI3Kδ over the other PI3K family

Table 4 | New drugs under development for adult primary warm autoimmune haemolytic anaemias

Drug name	Type of drug and target	Main cellular target	Way of administration	ClinicalTrials.gov identifier
Ianalumab	Human IgG1/κ mAb targeting BAFFR	B cells	IV every 4 weeks	NCT05648968
Povetacicept	Engineered modified TACI Fc domain targeting APRIL and BAFF	B cells	SC every 4 weeks	NCT05757570
Parsaclisib	Inhibitor of PI3 kinase PI3Kδ	B cells	Oral	NCT05073458
Rizalbrutinib	Bruton tyrosine kinase inhibitor	B cells; IgG-mediated FcγR activation and phagocytosis	Oral	NCT05002777
Obexelimab	Bispecific mAb that simultaneously binds CD19 and FcγRIIb	B cells, plasmablasts and CD19$^+$ plasma cells±B memory cells	SC weekly	NCT05786573
Daratumumab	IgGκ mAb targeting CD38	Plasma cells	Weekly IV or SC	NCT05004259
Isatuximab	IgG1 mAb targeting CD38	Plasma cells	SC	NCT04661033
Sovleplenib	Small molecule inhibiting Syk	Macrophages; B cells	Oral	NCT05535933
Nipocalimab	Humanized aglycosylated IgG1 mAb targeting FcRn inhibitor	Inhibition of the FcRn-mediated recycling of IgG, reducing pathogenic IgG levels	IV every 2 weeks	NCT04119050

BAFF, B cell activating factor; BAFFR, BAFF receptor; FcγR, Fcγ receptor; FcγRIIb, Fcγ receptor IIb; FcRn, neonatal Fc receptor; mAb, monoclonal antibody; IV, intravenous; SC, subcutaneous; Syk, spleen tyrosine kinase; TACI, transmembrane activator and calcium-modulating cyclophilin ligand interactor.

members[135]. Parsaclisib potently inhibits antibody-induced proliferation of human CD19$^+$ B cells. In addition, parsaclisib also inhibits B cell proliferation in response to multiple inflammatory stimuli (IL-4, lipopolysaccharides, CD40L, anti-CD40, IL-6 and BAFF) as well as the production of IL-17, IFNγ and IL-13 by naive T cells cultured under T_H17, T_H1 and T_H2 differentiation conditions, respectively. The results from a phase III trial in wAIHA are pending.

Obexelimab. Obexelimab is a bispecific mAb that simultaneously binds CD19 and FcγRIIb, resulting in the inhibition of B cells, plasmablasts and CD19-expressing plasma cells[136]. Based on this target, there is a rationale for testing its efficacy in wAIHAs.

Rilzabrutinib. Rilzabrutinib is a potent covalent reversible Bruton tyrosine kinase (BTK) inhibitor. BTK is a crucial signalling protein that links signals from the B cell antigen receptor to the activation, proliferation and survival of B cells[137]. Rilzabrutinib inhibits IgG-mediated FcγR activation, phagocytosis and inflammatory mediators in monocytes and macrophages. It has shown a promising efficacy in adult immune thrombocytopenia[137] and it is currently tested in wAIHA (Table 4). Other BTK inhibitors, such as ibrutinib (NCT03827603) and zanubrutinib (NCT05922839), are also being evaluated for treating refractory or relapsed wAIHAs[135].

Daratumumab. Daratumumab is an IgGκ mAb targeting CD38, a transmembrane receptor highly expressed in tumoural as well as normal plasma cells[138]. The rationale for testing the efficacy of daratumumab in AIHAs is to target autoreactive long-lived plasma cells, which are not effected by anti-CD20 mAbs[49]. Some case reports and small series have reported some efficacy of daratumumab for treating refractory CAD[138] or wAIHA secondary to Evans syndrome or following haematopoietic stem cell transplantation[139,140]. A multicentre retrospective study has also reported some data on the efficacy and safety of daratumumab monotherapy in a series of 19 patients with refractory wAIHA or CAD[141].

Isatuximab is another IgG1 mAb targeting a specific extracellular epitope of CD38 inducing the death of plasma cells by antibody-dependent cellular phagocytosis, ADCC and CDC; isatuximab has also been tested in wAIHA[135].

Sovleplenib. Sovleplenib is a small-molecule, potent, selective, oral inhibitor of spleen tyrosine kinase (Syk). Syk is a regulator of immune receptor signalling, which is important for B cell activation and autoantibody production. It is also involved in FcγR-mediated signalling, including cellular proliferation, differentiation, survival, immune regulation and cytoskeletal rearrangements during phagocytosis. Sovleplenib has shown a promising rate of durable response in adult immune thrombocytopenia in a phase II trial[142].

Nipocalimab. Nicopalimab is a mAb targeting the neonatal Fc receptor (FcRn)[135]. FcRn is a cytoplasmic receptor that is the central regulator of IgG homeostasis, rescuing IgG from lysosomal degradation, prolonging its half-life, and promoting its tissue penetration. Inhibition of the FcRn-mediated recycling of IgG leads to a reduction of pathogenic IgG levels. Several FcRn inhibitors have been developed for the treatment of autoantibody-mediated immune diseases such as myasthenia gravis[143]. Nipocalimab was granted a Fast Track designation in wAIHA by the FDA. Another FcRn inhibitor, orilanolimab, is also under development in wAIHA (NCT03075878).

Autologous CD19 CAR T cells. Following their approval as a third-line treatment of diffuse large B cell lymphoma, chimaeric antigen receptor (CAR) T cells have now been developed for severe or refractory autoimmune diseases, and the feasibility and efficacy following compassionate use of autologous CD19 CAR T cells have been reported in five patients with refractory SLE, two of whom also had haematological manifestations[144,145]. A phase I study aiming to treat individuals with AIHA or Evans has been begun (NCT06231368).

New complement inhibitors developed in wAIHA and/or CAD

Beyond sutimlimab, which has been approved by the FDA and the EMA for the treatment of CAD, and the new generation C1s inhibitor riliprubart, also developed for CAD, two other Ct inhibitors have been developed for AIHAs. The first is pegcetacoplan, a PEGylated pentadecapeptide that binds to C3 and its activation fragment C3b, controlling the cleavage of C3 and the generation of the downstream effectors of complement activation. It has already been approved in paroxysmal nocturnal haemoglobinuria[146], and the preliminary results from a phase II open trial in individuals with wAIHA or CAD are awaited. The second is ANX005, a humanized IgG4 anti-C1q recombinant antibody[135] for which the results of a completed phase II open-label study in individuals with wAIHA and Ct activation or mixed AIHA are also expected.

Quality of life

Measuring QoL is one of the hardest tasks for clinicians, including those dealing with AIHAs. A patient-reported outcome is any report of the status of the health condition (QoL and symptoms) of an individual that comes directly from the patient, without interpretation of the response by a clinician or anyone else. A UK group administered a semi-structured interview to 12 adult patients with AIHA and found that the disease had a significant effect on QoL for most patients[147]. On a 0–10 scale, in which 10 was the 'Worst I've ever felt', 77% of patients scored 5–10, and 31% scored 9 or 10. Patients described that the worst aspects of AIHA included the effect of investigation or treatment, symptoms (mainly fatigue), and anxiety over outcome. Multiple QoL domains were affected, including daily living, working, leisure and even sleep. Finally, the emotional well-being of both patients and their partners was adversely affected[147].

For cAIHA, an insurance registry-based study showed that patients were more likely than a control population to have medically attended anxiety and depression, to be prescribed antidepressants or psychotherapy, or to be hospitalized for an anxiety or depression episode[148]. In this setting, a specific questionnaire, the Cold Agglutinin Disease Symptoms and Impact Questionnaire (CAD-SIQ) was developed[149]. By asking 37 adults, common reported cAIHA-related symptoms were reactions to cold environments and fatigue, with a resulting negative impact on enjoyable activities (81.3%) and daily activities (75.0%). Through various rounds of interview, an 11-item score, including fatigue, cold sensitivity, dyspnoea, wearing extra clothing, limited physical, social and enjoyable activities, difficulty with usual activities, mood, frustration, and anxiety/stress, was validated. Beyond these initial efforts, most clinical trials with novel drugs for AIHAs evaluated fatigue as the main patient-reported outcome by using other surveys designed for other forms of chronic diseases, including cancer-related anaemia. The Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue instrument, designed to assess fatigue or tiredness and its effect on daily activities and functioning, has been evaluated in clinical trials. Several reports are now also available for sutimlimab in CAD[130,131,149] in which the baseline FACIT-Fatigue scores were similar to those observed

in patients with rheumatoid arthritis, advanced cancer-related anaemia or paroxysmal nocturnal haemoglobinuria. The drug improved FACIT-Fatigue score from a mean of 32.5 at baseline to 44.3 at 24 weeks. The reduction in fatigue was observed by the first week, with a mean difference of 7.2 points from baseline, maintained throughout the study and over 2 years of extension period[149]. Interestingly, improvements in Hb, bilirubin and FACIT-Fatigue correlated with complete complement inhibition. Anaemia-related symptoms of fatigue, weakness, shortness of breath, palpitations, rapid heartbeat, light-headedness and chest pain were also significantly reduced. As a further proof of concept, in the 9-week follow-up period after cessation of sutimlimab treatment, fatigue scores approached pre-sutimlimab values, indicating the recurrence of CAD after treatment discontinuation. Further scoring systems, such as the 12-Item Short Form Health Survey physical and mental component scores, the EuroQol Visual Analogue Scale, and the Patient Global Impression of Change and of (fatigue) Severity, were also used to assess the effect of sutimlimab on QoL of patients in the long-term CARDINAL and CADENZA studies[150,151]; all systems showed a sustained alleviation of CAD disease burden after long-term treatment.

Outlook

Challenges

Due to their rarity and despite their potential severity, AIHAs have long been left aside in terms of clinical and translational research. While certain gaps have been filled in translational research, mechanisms behind loss of tolerance towards self-RBCs are still far from being understood in wAIHAs. Since the early 2000s, the major advances made in the treatment of lymphomas and myeloma in specifically targeting B cells and plasma cells have subsequently benefited various autoimmune disorders, including wAIHAs. The recognition of the crucial role of the activation of the Ct system in the pathophysiology of a wide range of diseases as well as the development of potent Ct inhibitors in paroxysmal nocturnal haemoglobinuria paved the way for new therapeutic options for CAD with the approval of sutimlimab in this indication. Many other drugs targeting mostly autoreactive B cells are currently under development in AIHAs, which may further increase the rate of sustained remission. Challenges in the near future will be to identify some reliable predictive biomarkers of response to targeted therapies to move towards more personalized care and to make the management of relapsed or refractory AIHA cases more consensual. It is also important to create large prospective AIHA registries, especially for patients requiring transfusion support, older patients and individuals with significant comorbidities. Such registries would be helpful in providing a broader assessment and informing recommendations for the use of laboratory investigations and transfusion practice and could narrow the observed gap between guidelines and clinical practice.

In terms of clinical research, the priorities for the next 5–10 years will be to set up further randomized trials, especially in the field of wAIHAs, to assess the safety and efficacy of new promising B cell-targeted and/or plasma cell-targeted therapies with the aim of sparing the use of corticosteroids as much as possible. For the long term, the assessment of innovative therapies, such as CD19 CAR T cell therapy[148] or bispecific antibodies, in refractory AIHAs would be also helpful.

However, it must also be kept in mind that sophisticated diagnostic methods and advanced treatments are unfortunately still often not available or affordable in low-to-middle-income countries[152,153]. Overcoming the gap in real-world management of AIHA between high-income countries and low-to-middle-income countries will be one of the major challenges in this setting.

Finally, we are still in need of good biomarkers that could predict response to different lines of treatment and perhaps identify patients who will not achieve a durable response after first-line treatment.

Perspectives

In conclusion, much progress has been made into our understanding of the pathophysiology of AIHAs and in particular in the management of both wAIHA and CAD; however, the cost–benefit ratio of many new drugs remains an issue in many countries. Some unmet needs remain; first, there is currently no approved B cell-targeted or plasma cell-targeted therapy for the treatment of AIHAs and, for CAD, sutimlimab is either not available or not affordable in many countries. Secondly, even in countries where new drugs are available, the management of patients with refractory AIHAs remains challenging. In this evolving panorama, there is a real need for international guidelines on the diagnosis and treatment of primary AIHAs to aid physicians in choosing the best treatment option according to the type of AIHA and the patient profile.

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M.M. has received consultancy or advisory board honoraria and speaker's bureau from Alexion, Amgen, Grifols, Johnson and Johnson Novartis, Sanofi, Sobi and UCB. E.C. has received honoraria (advisory boards, speaker fees) from Novartis, UCB and Sanofi. W.B. has received consultancy or advisory board honoraria for Agios, Alexion, Amgen, Incyte, Novartis, Roche, Sanofi and SOBI; has received lecture fees or congress support from Alexion, Amgen, Novartis and Sanofi; and has received research support from Alexion. B.F. has received consultancy or advisory board honoraria and speaker's bureau from Agios, Alexion, Apellis, Janssen, Novartis, Roche, Samsung, Sanofi and Sobi.

Informed consent
The authors affirm that human research participants provided informed consent for publication of the images in Fig. 2.

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